Biochemistry 661
Nucleic Acids, Module 1
Exam 1 (100 points total)

You have 60 minutes for this exam.

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

Explanations should be concise and clear. I have given you more space than you should need. There is a
extra space on the last page if you need it.

You do not need a calculator for this exam, and no other study aids or materials are permitted.

Generous partial credit will be given, i.e., if you don’t know, guess.

Honor Pledge: At the end of the examination time, please write out the following sentence and sign it, or
talk to me about it:

“I pledge on my honor that I have not given or received any unauthorized assistance on this examination.”

1. (16 pts) You have sequenced an RNA isolated from an extract that helps carry out splicing. You would
like to know its secondary structure. Describe how you would proceed. Additionally, you would like to
identify base pairing interactions between your new RNA and potential splicing partners. What technique(s)
could allow you to catch them? (More space on next page if you need it.)

1. Predict 2'S using mfold or similar program - based on
selecting minimum free energy structure from nearest neighbors

2. Make variant RNA's that have correlative invariants to
preserve predicted base pairing - see if the melting profile
changes

3. Use ss-specific or ds-specific nucleases or perform
STEAP analysis to analyze 2'S experimentally, directly

To find partners:

1. Attempt psoralen crosslinking and map with reverse transcriptase
2. Make variants that disrupt or preserve putative contacts,
   see if function changes
2. (6 pts) Why don’t sequence-specific RNA binding proteins recognize a fully double-stranded A-form RNA helix?

- The deep major groove is not accessible to protein, so the sequence-difference 11-bond surface of the major groove face cannot be read out to distinguish different sequences.

3. (8 pts) The MAGE paper described a technique that allows rapid genome engineering/evolution. Briefly describe the advantages of this technique as opposed to either traditional cloning or random mutagenesis in generating new or enhanced phenotypes.

- The technique allows creation of combinatorial genetic diversity. For $n$ target sites there are $2^n$ combinations of $+1$ mutations. For $n = 1$ traditional cloning is fine, but for $n = 20$ it is impossible to explore many more variants.

- But after all we are limited by the # of cells to screen, and random mutagenesis could make an essentially infinite number of different cells, but the vast majority of the mutants are at places we don’t care about. MAGE makes changes at targeted locations so new phenotypes are much more likely to appear and be understandable if they do.
4. (18 pts) Here is the structure of L-2'-deoxyribose vs. the usual D-2'-deoxyribose.

Draw an A-T base pair with L- instead of D-deoxyribose, including both sugars and the four pendant phosphates (three of them just as P's with a circles around them). Sketch the B-form double helix that would result, with strand directions indicated.

Score for the page 18
5. (7 pts) When we talked about DNA flexibility I said, “There is no ‘the structure’ there.” What does this mean? Define a “persistence length.”

5' DNA structure is statistical— for long DNA we think about an ensemble of possible structures, not one unique structure.

5' Persistence length = a measure of how long a segment is needed to “forget” initial dexterity based on $\langle \mu^2 \rangle = \frac{\epsilon}{\gamma}$.

5' or distance, on average that the end is displaced in the initial direction.

$\langle \mu^2 \rangle = \frac{\epsilon}{\gamma}$.

6. (12 pts) The T4 ligase mediated cyclization assay provided a conclusive demonstration that DNA is a double helix in solution. The Shore and Baldwin curve of cyclization probability vs. DNA length is sketched here.

Label the part of the curve that shows rigid rod behavior and the part that looks like random coil.

Sketch on the figure how we can measure the DNA helical repeat from this curve.

On the curve to the right, sketch what the curve would look like if the DNA were significantly stiffer, in terms of torsion only.
7. (15 pts) The portion of the table shown from Borer et al., 1974, provides evidence that a model for hybridization thermodynamics based solely on base composition is inadequate. What is the reasoning for this conclusion? They introduced a nearest-neighbor model for thermodynamics that has subsequently been elaborated by many groups. What is the basic idea of nearest-neighbor thermodynamics? Any scientific theory must be falsifiable (if a theory isn’t falsifiable, we call it religion). How could the nearest-neighbor theory have been falsified?

<table>
<thead>
<tr>
<th>Molecule†</th>
<th>$T_m$ (°C) at‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 μM</td>
</tr>
<tr>
<td>$A_4CG + CGU_4$</td>
<td>-13.9</td>
</tr>
<tr>
<td>$U_2CGA_2$</td>
<td>1.6</td>
</tr>
<tr>
<td>$A_2CGU_2$</td>
<td>10.8</td>
</tr>
<tr>
<td>$A_4G_2 + C_2U_4$</td>
<td>14.0</td>
</tr>
<tr>
<td>$A_4CGU_2$</td>
<td>19.6</td>
</tr>
</tbody>
</table>

Note: *C* - type in original

- $U_2CGA_2$ and $A_2CGU_2$ have the same composition of $U’s$, $C’s$, $A’s$, and $G’s$, but their $T_m$’s differ by 10°.

In fact all of these oligo have 4 A:U and 2 G:C pairs but their $T_m$’s are very different. [I should have shown the $A’s$, $C’s$, and $G’s$ – the latter two are the actual new derived quantities.]

- The idea of the NN model is that nearest neighbors or dinucleotides make independent contributions to the overall degree of hybridization.

- If the NN model had failed to predict the $T_m$’s of new sequences, we would know that long-distance considerations were necessary. Or – if all of the same NN’s but different sequence have different $T_m$’s. In fact this does occur and requires a small correction.
8. (18 pts) Negatively supercoiled DNA can extrude quadruplex segments of DNA. Give a sequence that could form a quadruplex. What structure can the other strand form? Sketch a plasmid with and without an extruded quadruplex to explain why negative supercoiling has this effect. Give estimates for before-and-after ΔTw and ΔWr.

- AAA GGG ATT GGG ATT GGG ACAT
- 4 G segments separated by at least 3 nt
- The other strand can form an ε-motif +3
- Q-plex unwound
- Plasmid

ΔWr ≈ 0.8 x -0.06 = 0.048 N/m
ΔTw ≈ 2.350 N/m
h = 10.5 mm
6Δwr = -0.05 x 300 = -15
6Δtw = -0.0125 x 300 = -3.75
6Δk = 300
-0.06 x 300 = -18 turns
+3 for reasonable estimates

Score for the page /18